

Effect of Increasing Doses of BerberisLycium on Alkaline Phosphate in acetaminophen induced liver damage in Mice.

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ABSTRACT

Background: Liver disease is a common health problem. Conventional drugs not only cause economic burden but also have many side effects. In medical science herbal remedies have shown beneficial in curing many medical disorders. Berberislycium herbal plant has been used for treating many health related problems. The current study is conducted to observe the hepatoprotective effect of this plant extract on drug induced liver damage.

Objectives: To study the hepatoprotective effects of Berberislycium on Alkaline phosphatase in acetaminophine induced liver damage in mice.

Methods: This experimental study was carried out at Peshawar Medical College Warsak Road Peshawar. Twenty adult Swiss albinomice were selected and divided into four groups with five mice in each group. All groups were made hepatotoxic with acetaminophen 250mg/kg body weight intraperitoneal injection. Group I received acetaminophine only, Group II-IV (E1-E3) were given increasing dose of extract 100mg/kg, 200mg/kg and 400mg/kg body weight respectively. Serum blood samples were taken for Alkaline Phosphatase ALP liver enzyme. Hepatoprotective effect with increasing doses of extract was seen.

Results: ALP Alkaline Phosphatase of experimental group I was 518 ± 41.2 U/L Alkaline Phosphatase levels of experimental groups II-IV were 262 ± 77.8 , U/L 184.6 ± 46.8 U/L and 155 ± 34.4 U/L respectively indicating that increasing dose of plant extract lowers Alkaline phosphatase levels

Conclusions: Herbal plant Berberis Lycium has shown hepatoprotective effect on liver enzymes and increasing doses of extracts have reduced the level of alkaline phosphatase to normal. This herbal plant can be used as a hepatoprotective agent.

Keywords: Berberis Lyceum, extract, acetaminophine, Alkaline Phosphatase

INTRODUCTION

Since ancient times human health and its related diseases have been a subject of man's primary concern. Herbal plants were used in the treatment of various ailments because of low toxicity and fewer side effects. Various experiments with plants, humans were eventually able to establish empirical system of medicine. According to WHO about 80% of the world population rely mostly on traditional medicines.¹ Most of the therapeutic effects of plants are seen due to the presence of active principles in them. A large number of crude plant extracts are now being utilized in naturopathic remedies in addition to the purified natural substances.² Liver disorders are common health problem. These are traditionally treated by medicinal plants especially in rural areas where they are widely available. Liver causes detoxification and metabolism of drugs, chemicals, metals, bilirubin, steroid hormones and amino acid.^{3,4}

Acetaminophen commonly used as an analgesic and its over dosage either intentionally or unintentionally remains the most common cause of fulminant hepatic failure with mortality rate of 90%. This is because of higher dose acetaminophen's conversion to extremely toxic intermediate N-acetyl-p-benzoquinone imine (NAPQI) by several P450 cytochrome enzymes.⁵

Berberislycium (family: Berberidaceae) a herbal plant mostly found in the Himalayan region. In Pakistan, it grows in Baluchistan, Khyber-Pakhtunkhwa, Punjab and Azad Kashmir at a height of 900 to 2900 meters.⁶ We used Leaves of Berberislycium, they are of bright color, oblong-ovate shape, thick and entire toothed. Berberislycium leaves due to their antioxidant properties are used for the treatment of various liver disorders. These leaves were used as a tea substitute and were used to normalize the deranged liver function.⁶ Berberislycium has been used as antibacterial, anti-carcinogenic, carminative.⁶ It is also used for various endocrine abnormalities, anti-diabetic and anti hyperlipidemic.⁷ It is also used for treating various central nervous system disorders.⁸ The hepatoprotective properties of *Berberislycium* need to be scientifically proved to make commercial drugs from this plant to benefit humans.

AIMS AND OBJECTIVES

To determine the hepatoprotective activity of Berberislycium on acetaminophen-induced liver damage in mice

Study Design: Quasi-experimental Study

Settings: Peshawar Medical College, Peshawar

Duration of study: six months

Sample size: Twenty Swiss albino mice

Sample technique: Random

Inclusion criteria :

Healthy young adult mice of both genders, with weight between 22-35 grams and age 8-12 weeks

Exclusion criteria : Pregnant female mice, unhealthy mice.

METHODS AND MATERIALS

The Plant *Berberis lycium* was selected on the basis of its traditional use and its phytochemical profile. Leaves were collected, washed and placed in shade to dry. After 20 days dried leaves were grounded to powder form. Powdered leaves were soaked in 8 liter of 99.9% methanol for 7-10 days. This paste was shaken regularly on alternate days. On 10th day the solution was filtered with the help of muslin cloth. Filtrate was collected in conical flasks and were allowed to evaporate with the help of the rotary evaporator. After 3 days a brownish green paste was obtained, placed in a dish kept in water bath for 4 days. A thick jell paste was obtained which was stored in amber colored bottle for experimental purpose.

Mice were kept in cages in animal house of Peshawar Medical College under standard environmental conditions. Twelve hours dark and light cycle was maintained. Temperature was kept at $25 \pm 2^\circ\text{C}$. They were acclimatized for about 10 days.⁹ Mice were fed on standardized pellet diet with water ad libitum.⁹

Twenty mice were divided into four groups with (n=5) five animals each group.

Group I. Control Group. Intraperitoneal injection of Acetaminophen 250 mg/kg body weight was given

Group II –E1: Given Plant Extract 100 mg/kg body weight and then after 3 hours Acetaminophen 250 mg/kg/body weight was given

Group III – E2: 200 mg/kg body weight Plant extract was given, after 3 hours Acetaminophen 250 mg/kg body weight was given

Group IV-E3: Received Plant Extract 400 mg/kg body weight followed by Acetaminophen 250 mg/kg body weight was given after 3 hours.¹⁰

After ten days the mice were fasted for 12 hours, anesthetized with light chloroform and sacrificed. Blood was collected by direct puncture in to aorta and samples were preserved in tubes for serum preparation¹⁰. Blood was centrifuge for the separation of serum at the rate of 4000 cycles/min for about 20 minutes. Liver Function tests i.e., Alkaline Phosphatase was evaluated through standard operating procedures.¹¹

RESULTS

The data was collected and a series of statistical tests were performed. In the test of normality the whole data was found normally distributed. For significant results among different variables student t test was applied through SPSS version 20.

Group I acetaminophine control group. Group II-E1, received extract 100mg/kg body weight with acetaminophine. Group III-E2 was given extract 200mg/kg body weight with acetaminophine. Group IV received acetaminophine with extract 400mg/kg body weight. Alkaline Phosphatase levels in Groups.

Different doses of plant extract were given and results were obtained. In Group II- E1, (100 mg/kg) ALP was 262 ± 77.8 IU/. Group III-E2, (200 mg/kg) ALP was

184.6 ± 46.8 IU/L and in group IV- E3, (400 mg/kg) Alkaline Phosphatase was 155 ± 34.4 IU/L.

Figure 2 shows increasing doses of *Berberis Lycium* reduces serum Alkaline Phosphatase levels. This proves the hepatoprotective effect of *Berberis Lycium*.

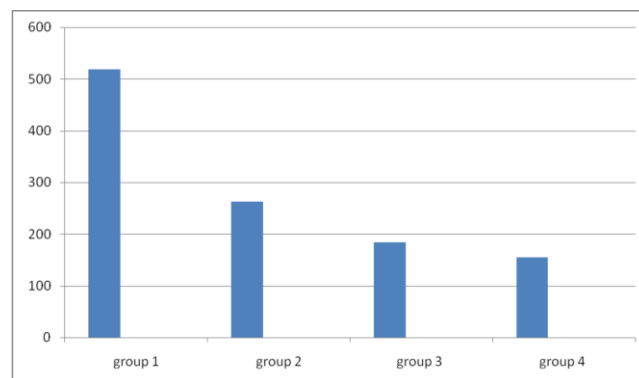


Figure 1 Alkaline Phosphatase

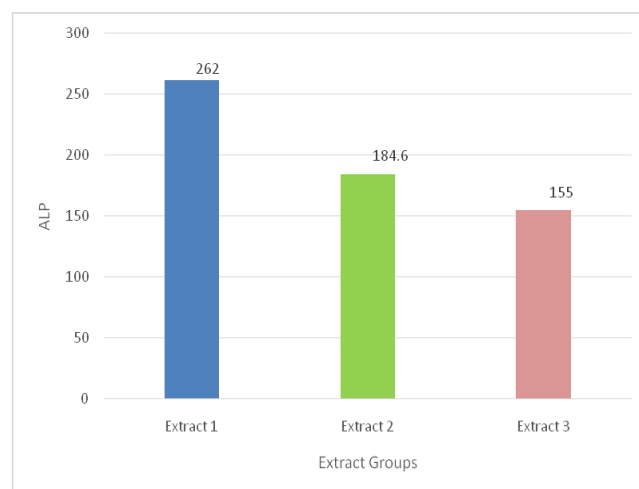


Figure 2. Experimental groups for Alkaline Phosphatase

DISCUSSION

Drugs side effects and toxicity has been a matter of serious concern in medical science. Drugs reported to be toxic have been de-registered and removed from the market. Liver being a major site of metabolism for many xenobiotics experiences the effect of injury the most. Many herbal plant extracts have shown their antioxidant and hepatoprotective effects¹².

Berberis lycium is one such plant which has been revealed in folk medicine for its healing properties including beneficial effects on liver. This became the motivation for the present study because no such scientific study has been previously done to prove the claims about the usefulness of *Berberis lycium* in liver ailments as antioxidant and as hepatoprotective¹³

Twenty mice were selected in our study as animal model, hepatotoxicity was induced with acetaminophen. Mallhiet.al in his study used Paracetamol to induce liver toxicity and studied the hepatoprotective effect of herbal plant on liver.¹⁴ In present experimental study, all groups showed acetaminophen-induced hepatotoxicity,

Berberislycium extract with increasing doses reduced the elevated levels of serum Alkaline Phosphatase levels most significantly ($p < 0.000$).

Many research scholars have studied antioxidant properties of herbal plants for protecting various organs from oxidative stress. Rafiq et.al used bark of BerberisLycium in his research against isoniazid-induced liver toxicity and found the hepatoprotective activity of the plant. This study is in consistence with our study and we used leaves of the plant instead of bark.¹⁵

Khan et.al used mice in his study and also used extracts of three different plants including BerberisLycium against CCl₄-induced liver toxicity. He concluded that a mixture of these plants extract not only reduced elevated liver enzymes but also reduced the process of necrosis in liver cells.¹⁶ Their work supports our study for contributive hepatoprotective role of Berberislycium in combination of three herbs.

Purvika et.al conducted a study on rats and studied the hepatoprotective effect of BerberisLycium on carbon tetrachloride induced liver toxicity. Elevated liver enzymes were reduced to normal after treatment with Berberis lycium.¹⁷ The contradiction to our study is that in their study Purvika used rats rather than mice as their experimental model. Furthermore he induced hepatotoxicity in rats by giving carbon tetrachloride solution and then Berberislycium was given.

A similar study was conducted by Girish et.al also used mice as their experimental model and observed beneficial results with different combination of herbal plants.¹⁸ Ur Rehman et.al studied the antioxidant effect of BerberisLycium and concluded that this plant has a beneficial role in protecting organs against oxidative damage.¹⁹

In light of above findings of our study and its concordance with the previous studies, it is indicated that the methanolic extract of leaves of Berberislycium in higher doses significantly ($p < 0.000$) reversed the inflammatory changes in damaged liver of mice BerberisLycium due to its easy accessibility, economical factors and edible nature has healing potential in liver ailments. However, to add it to the list of hepato protective drugs, further research to be done to find its active ingredients, their pharmacodynamics, pharmacokinetics and toxicity to be evaluated.

CONCLUSION

Data analysis of our study conclude that Berberislycium has hepatoprotective properties. This herbal plant has significantly reduced the elevated levels of ALP Alkaline Phosphatase to lower levels. The increasing doses of plant extract brought the liver functions to nearly normal. The study results support the traditional use of this herbal plant.

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